A STUDY OF DORMANCY AND GERMINATION OF SEEDS OF CERCIS CANADENSIS[1]

By Michel Afanasiev

Associate horticulturist Oklahoma Agricultural Experiment Station

ANATOMY OF THE SEED

The seed of redbud (*Cercis canadensis* L.) is of the albuminous type. It has a very thin but strong seed coat, a considerable amount of perisperm and endosperm tissue, and, at the time of maturity, a well-developed embryo (fig. 1).

The seed coat (*a*), ranging in color from light tan to dark brown, is composed of small, thick-walled cells impermeable to water. These cells are cylindrical in form with the long axis perpendicular to the surface of the seed, and are commonly referred to as Malpighian cells. The perisperm (*b*), found immediately under the seed coat, is composed of fairly small, thick-walled cells. Unlike those of the endosperm, these cells are arranged in more or less regular rows, both radially and tangentially. The endosperm cells are thin-walled and irregular in shape and arrangement (*c*). The presence of a distinct perisperm in tree seeds is rather unusual, this tissue being normally either entirely absent or so similar in structure to the endosperm that it can hardly be differentiated from it. The mature embryo (*d*), occupying the central cavity of the endosperm, is about 4 mm. long with cotyledons 2½ to 3 mm. wide. In a ripe seed it is fully developed, and all its parts are completely differentiated. The seed is oval in form, somewhat flattened, and is borne in a pod varying in length from 6 to 8 cm.

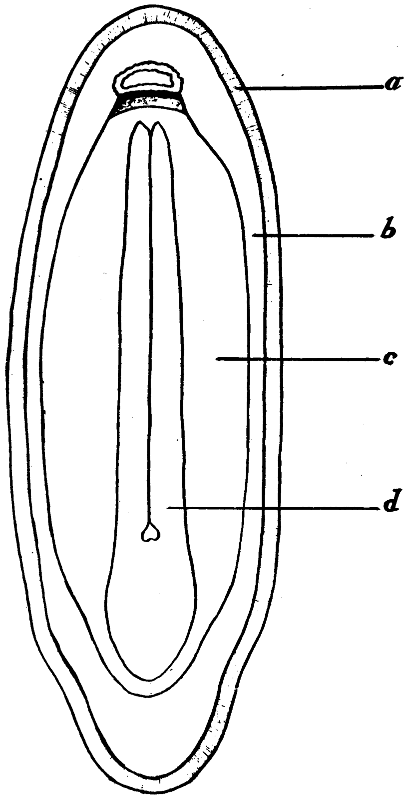

FIGURE 1.—Longitudinal section of redbud seed: *a*, Seed coat; *b*, perisperm; *c*, endosperm; *d*, embryo.

In Oklahoma the seed ripens at the end of August or the beginning of September, but remains on the tree throughout the winter. While still on the tree the seeds are subject to injury and destruction by

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weevils. When collected in the late fall or winter the seeds were invariably worthless, being heavily infested with insects. In nature the seeds which escape injury and fall to the ground usually remain dormant for several years.

MATERIALS AND METHODS

Seeds of the redbud used in this investigation were either collected in Oklahoma (lots 39-0, 39-B, 39-C, 40, 41), mostly in the vicinity of Stillwater, or purchased from a commercial seed dealer in Boston, Mass. (38-A, 38-B). A few experiments, chiefly for the verification of the original results, were performed with seeds collected in Kansas (39-K). With the exception of a few small lots collected between June 28 and August 25 of 1939 and used for the specific purpose of determining some properties of immature seeds, all seeds were collected when completely ripe.

Seeds after being collected and air-dried for several days were stored in glass or metal containers, usually at a temperature ranging between 35° and 50° F. Germination tests, as a rule, were carried out on moist cotton in Petri dishes. However, in some instances indicated in the text, the seeds were tested also in soil, either in flats or in the nursery seedbeds; and in a few cases, tests were made in Erlenmeyer flasks as described elsewhere in this paper. Only sound seeds were used in all tests. These were easily separated from the decayed, empty, or otherwise poor seeds after a treatment with concentrated sulphuric acid followed by washing and soaking seeds in cold water. Poor seeds failed to absorb water in large quantities and usually remained thin and shriveled, while sound seeds imbibed water freely and swelled to several times their original volume in 8 to 12 hours.

The stratification medium used throughout the investigation was granulated peat moss purchased from a chemical company in Kansas City, Mo. When storage at low temperatures was needed, the samples were held either at 37° to 45° F. in an electric refrigerator or in a cold room in which the temperature varied irregularly from 35° to 53° F. with an average of 44°.

It is estimated that more than 50,000 seeds of redbud were used during the investigation.

CAUSES OF DORMANCY AND SEED TREATMENTS

CAUSES OF DORMANCY

Two factors are responsible for delayed germination of redbud seed. One is restriction of the intake of water caused by the nature of the seed coat, a characteristic very common in seeds of legumes (*6, 9*).[2] Another lies in the embryo, which fails to grow even when the seed absorbs a large quantity of water. Several samples of sound fully swollen redbud seeds remained under germinative conditions in the writer's laboratory for as long as 716 days without germinating. Dormancy of the embryo is responsible for the failure of fully swollen seed to germinate. Excised embryos kept under conditions favoring growth usually exhibited phototropism and geotropism,

[2] Italic numbers in parentheses refer to Literature Cited, p. 420.

produced chlorophyll, but failed to grow and produce seedlings. Only in a few instances have excised embryos been observed to grow to a considerable size (*2*). Fiom the practical point of view, redbud embryos should be considered dormant and the seed treated accordingly.

SEED TREATMENTS

Two separate problems are involved in forcing the germination of redbud seed: (1) Modification of the seed coat in order to permit absorption of moisture which is necessary for afterripening; and (2) afterripening of the seed. The treatment of redbud seeds prior to germination does not differ from that of other seeds having similar causes of delayed germination.

The impermeable character of the seed coat can be modified by any of the three methods commonly used on the so-called "hard" seeds in commercial and experimental work (*8, 12, 13*). The standard treatment of seeds with concentrated sulfuric acid (sp. gr. 1,842) proved to be particularly effective, and once the requirement for any one lot had been determined, acid treatment was used throughout the period of investigation whenever the seed coats had to be made permeable to water. As would be expected, individual lots of seeds varied somewhat in the intensity of acid treatment required. No attempt was made to standardize the temperature at which the treatments were carried out. The temperature varied between 70° and 85° F.

At the beginning of the investigation, the optimum period of acid treatment was determined by germination tests of seeds treated for various periods. Later, however, the ability of treated seed to absorb water and the freedom from injury of treated seed were used as the criteria of the proper length of the treatment. Undertreated seeds when placed in water do not absorb it and their volume remains unchanged. Overtreated seeds, on the other hand, absorb water freely and swell to several times their original volume, but also exhibit definite sign of overtreatment in the form of "burned off," light-colored spots appearing on the seed coat (fig. 2). Correctly treated seeds when placed in water swell markedly in 8 to 12 hours, yet their coats remain smooth and intact. Owing to a certain degree of structural variation in seed coat of the same species, it is impossible to determine a "perfect" treatment for any lot of seeds. Any treatment that will render every seed permeable to water will result in overtreating at least a few of the seeds. Table 1 shows the proportions of swollen and "spotted" (injured) seeds caused by treatment with concentrated sulfuric acid followed by soaking in water.

TABLE 1.—*Proportion of swollen and injured seeds caused by acid treatment of various lengths of time; lot 39–K*

Condition of seeds	Not treated	Acid-treated for—				
		10 minutes	20 minutes	30 minutes	40 minutes	60 minutes
Percent of seeds swollen	0	5	60	100	100	100
Percent of seeds "spotted"	0	5	15	35	85	90

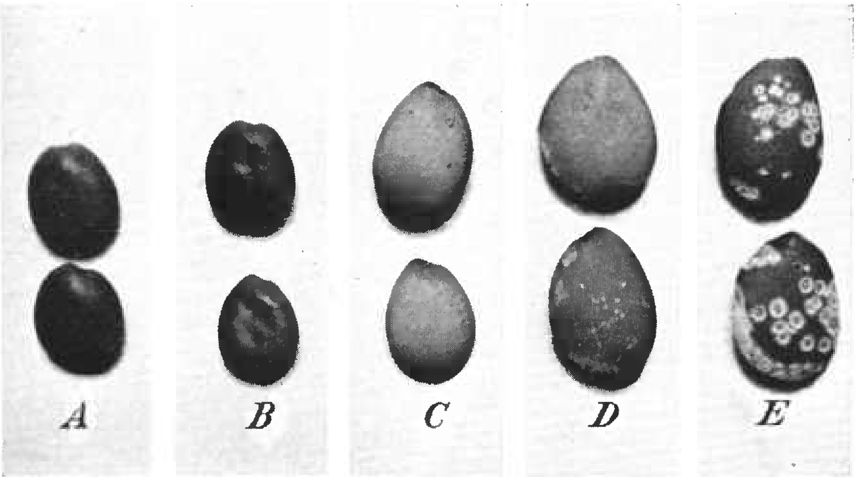

FIGURE 2.—Redbud seeds after various periods of sulfuric acid treatment followed by soaking in cold water for 24 hours (lot 39–0): *A*, Neither treated nor soaked; *B*, not treated but soaked in water; *C*, acid-treated 10 minutes; *D*, acid-treated 20 minutes; *E*, acid-treated 40 minutes.

In determining the optimum period of treatment the question arises as to whether it is more desirable to obtain permeability in all seeds and have some seeds overtreated or to leave part of the seeds with the seed coats still impermeable to water but avoid as much as possible the injury to the seed coats from overtreating. It should be stated that a slight injury to the seed coat does not affect the soundness of the kernel or interfere with the afterripening and germination of the seed, although more serious injury may pave the way for its decay and ultimate destruction. The shortest period of treatment that causes all seeds to absorb water does not usually result in a critical injury to any of the seed and should therefore be considered as the optimum.

The needed length of treatment as determined by the ability of seed to absorb water can be ascertained in a few hours. The optimum treatment of each lot used in this investigation was found by treating small lots of seed for from 10 to 60 minutes. Seeds less than 1 year old responded best to treatment for from 25 to 30 minutes. With the lengthening of the storage period between the time of collection and the time of treatment (age), the response of the seed to acid treatment became slower. The most effective treatment (30 minutes) during the first winter after collection was not sufficiently long when applied to the same lot of seed after a storage of 15 months, as is indicated by the accompanying tabulation:

Lot No.	*Optimum period of acid treatment—minutes*
38–B	30
39–K	25
39–O	30
38–B (after 15 months of dry storage)	45

Hot or boiling water was another medium that was effective in rendering the seed coats permeable to water. Only a small portion of the seeds of lot 38–B soaked in water heated to 180° F. were made permeable to water, whereas submerging them in boiling water for ¼ minute, ½ minute, 1 minute, and 2 minutes resulted in absorption of water by 62.5, 86.5, 97.5, and 97.5 percent of the seeds, respectively. Seeds of the same lot properly treated with concentrated sulfuric acid (30 minutes) responded 100 percent to the treatment (table 2).

TABLE 2.—*Comparative effectiveness of hot water and sulfuric acid treatments on permeability of seed coat to water, lot 36–B*

Treatment	Percent of seed swollen after 35 days in stratification	Percent germination after indicated days in stratification		
		7	21	35
Boiling water, ¼ minute	62.5	0.5	12.0	28.5
Boiling water, ½ minute	86.5	1.5	26.0	44.0
Boiling water, 1 minute	97.5	2.5	18.0	47.0
Boiling water, 2 minutes	97.5	4.5	6.5	4.5
H_2SO_4, 30 minutes	100.0	4.0	60.5	[1] 83.0
Hot water (180° F.)	Few	0	0	1.0

[1] Stratified 28 days.

Use of boiling water even for 1 or 2 minutes has never given as favorable germination results as did the treatment with concentrated sulfuric acid. Although boiling the seed for ½ minute or less did not sufficiently modify the seed coats in all seeds, boiling for 1 or 2 minutes damaged embryos in at least some of the seeds. Lengthening the period of boiling-water treatment from ¼ to ½ minute or to 1 minute increased the percentage of seeds rendered permeable to water as well as the final percentage of germination. Boiling the seed for 2 minutes reduced germination markedly. Attempts to modify the seed coat by ether, which was found to be effective in increasing the percentage of germination of seeds of *Robinia pseudoacacia* (*9*), proved to be unsuccessful with redbud. Mechanical scarification was highly effective on redbud when each individual seed was scratched, cracked, or sandpapered. No mass treatment of seeds by a mechanical scarifier was tried.

Since the impermeability of the seed coat to water is not the only cause of the delayed germination of redbud seed, modification of seed coat alone is not sufficient to break its dormancy completely: stratification of the seed is also necessary.

Many thousands of seeds of several crops were subjected to stratification during this investigation. The results of seven series of tests (typical for the redbud seed) are presented in table 3. The stratification requirements of individual lots varied considerably, as did those of individual seeds of any one lot. Yet despite these variations two definite conclusions concerning stratification requirements of redbud seeds appear to be justified.

(1) All seeds of redbud must complete afterripening and cannot be forced to germinate unless previously kept under conditions favoring this process. Stratification at 35° to 45° F. favors afterripening.

(2) A stratification period of 5 to 8 weeks at 35° to 45° F. is required for the completion of afterripening of 90 percent or more of seeds in practically all instances. In individual cases, germination of more than 80 percent was secured after less than 4 weeks of stratification (lots 38–B and 39–0) and in one lot (38–B) the seeds germinated to the extent of 94 percent after only 22 days of stratification.

TABLE 3.—*Effect of the length of the stratification period on afterripening of acid-treated [1] seeds of redbud*

Days in stratification	Percent germination in lots—[2]						
	38–A	38–B	38–B_1	38–B_2	39–K	39–O	39–C_1
0					0	0	0
5			4.0	0			
7					15.0	4.0	
8		28.0					
11			4.0	3.0			
14							10.0
15		50.0	4.0	3.0			
21					42.5	60.5	13.0
22		94.0					
25	40.0		10.0	17.0			
28					79.5	83.0	22.0
29	70.8	88.0					
30			14.0	11.0			
35	50.0	96.0	34.0	34.0	97.0		31.0
40			26.0	50.0			
42					99.0		80.0
46		92.0	55.0	89.0			
49							90.0
50			84.0	95.0			
55	47.0	81.0					
56	100.0	88.0			100.0	96.0	
60			88.0	95.0			
63					99.5	95.0	
64							97.0
65			53.0	72.0			
70			23.0	60.0	98.5	94.5	
75			16.0	45.0			
80							
81			56.0	86.0			
84							
86			57.0		99.0	97.0	
91					100.0	99.5	
92							
93			9.0	1.0			
98						100.0	
100				3.0			
105						99.5	

[1] Seeds treated 25 or 30 minutes according to the requirement of individual lots.
[2] The dates in 1939 on which first germination tests were started for the different lots follow: 38–A, Feb. 27 38–B, Mar. 15; 38–B_1, June 27: 38–B_2, June 27; 39–K, Nov. 10; 39–0, Nov. 10; 39–C_1, Oct. 4.

Extension of the stratification period for a few weeks beyond that needed for the completion of afterripening does not seem to interfere seriously with the ability of the seed to germinate. Therefore it appears safe to delay planting of afterripened seed should such delay become necessary. The only two samples of seeds in which germination was reduced with the extension of the stratification period were those stratified on June 22, 1939 (lots 38–B_1 and 38–B_2). Since germination of afterripened redbud seed is adversely affected by extremely high temperature (see table 7), it is very probable that this factor was responsible for the reduction in germination of seeds of these lots rather than the extension of stratification beyond the period 7 to 9 weeks, at which time germination reached its maximum. Germination tests of these two lots were carried on into the late summer when the temperature in the laboratory often exceeded 90° F., whereas the temperature of germination of other lots varied between 70° and 85°.

In addition to the sulfuric acid and stratification method of germinating redbud seeds, various other "forcing" methods and agents were tried. A summary of these treatments and of the results obtained is presented in table 4. For the purpose of comparison, seeds stratified after a treatment with sulfuric acid are also included in the table.

None of the treatments were so effective in afterripening of redbud seed as the combination of proper sulpuric acid treatment and stratification.

TABLE 4.—*Effect of various treatments on afterripening and germination of redbud seeds: lot 38–B*

Treatment	Highest germination	
	Percent	Period in days
None	0	716
H_2SO_4 30 minutes	10.0	365
Stratification 125 days	1.0	90
H_2SO_4 30 minutes and O_2 100 percent for 7 days	0	[1] 7
H_2SO_4 60 minutes, followed by freezing for 21 days	0	[2] 18
H_2SO_4 30 minutes, followed by stratification at weekly alternations of temperature 35°–75°	3.0	50
H_2SO 60 minutes, and concentrated HNO_3, 1 minute	25.0	24
H_2SO_4 15 minutes, followed by stratification for 36 days (41° F.)	46.0	18
H_2SO_4 30 minutes, followed by stratification for 35 days (41° F.)	96.0	8
H^2SO_4 60 minutes, followed by stratification for 36 days (50° F.)	29.0	39
H_2SO_4 60 minutes, followed by stratification for 27 days and soaking in 1 : 5,000 formic acid for 20 hours	38.0	15
H_2SO_4 60 minutes, followed by stratification for 27 days and soaking in 1 : 5,000 NaOH for 20 hours	56.0	15
Boiling water 2 minutes followed by stratification for 35 days (41° F.)	4.5	33

[1] In oxygen.
[2] All dead.

Substitution of freezing for stratification not only failed to bring about afterripening but actually injured the seed. Injury to the embryo caused by mistreatment, mishandling, or by application of certain chemicals always became evident in a few days after the seeds were placed under conditions favoring germination. The loss of viability of injured seeds manifested itself first in a rapid development of fungi on and around the seeds and then in a complete decay and destruction of the affected seeds.

Use of concentrated nitric acid to supplement sulfuric acid treatment caused germination of 25 percent of seeds without stratification but damaged the remainder of the lot. Application of weak solutions of formic acid and sodium hydroxide markedly hastened germination as compared with the application of H_2SO_4 alone, and increased the total percentage of germinated seeds. Germination of seeds not treated with sodium hydroxide was 29 percent as compared to 56 percent for seeds soaked in sodium hydroxide for 20 hours. Increasing the oxygen concentration of the air to 100 percent did not produce as good germination results as stratification, although it had a very pronounced stimulative effect on germination of afterripened seeds, as will be shown later.

An attempt was also made to hasten afteripening in stratification by holding acid-treated seeds in pure oxygen before placing them in stratification. Such treatment not only failed to bring about the desired result but actually lowered germination. After 6 weeks of stratification, 98 percent of seeds not treated with oxygen had germinated while seeds held for 24 hours in pure oxygen prior to stratification germinated to the extent of only 36.5 percent (table 5). A similar effect of the increased oxygen supply on afterripening has been observed in the case of red cedar seeds (*3*, *10*).

TABLE 5.—*Effect of vitamin B_1 and pure oxygen on afterripening*

Treatment	Percent germination after stratification for—		
	2 weeks	4 weeks	6 weeks
H_2SO_4, 30 minutes; vitamin B_1, 24 hours; stratification	18.0	38.5	99.5
H_2SO_4, 30 minutes; pure oxygen, 24 hours; stratification	2.0	6.5	36.5
H_2SO_4, 30 minutes; soaking in water, 24 hours; stratification	15.5	27.5	98.0

In view of frequent claims of the extraordinary stimulative effects of vitamin B_1 on plant growth, a few tests to obtain information as to its value were carried out. One of these dealt with the effect of the vitamin on afterripening. Seeds of lot 39–C, after being treated for 30 minutes with concentrated sulfuric acid, were soaked for 24 hours in a solution containing 4 mg. of vitamin B_1 in a liter of water. After the completion of the treatment the seeds were stratified and the rate and degree of afterripening were compared with those of seeds which were treated with sulfuric acid and then stratified without being soaked in the vitamin solution. In each test germination of vitamin-treated seeds was slightly higher than that of untreated seeds (table 5). The differences in total germination, however, were too small to be significant.

MICROCHEMICAL TESTS

Observations on stored food and three oxidizing enzymes in the seed were made by means of the following tests and reagents: Protein-biuret reaction, fats-Sudan IV; reducing sugars-Flückiger reaction; starch-iodine potassium iodide solution; oxidase-alcoholic solution of gum guaiac; peroxidase-alcoholic solution of gum guaiac and 3-percent solution of hydrogen peroxide; catalase-3-percent solution of hydrogen peroxide neutralized with calcium carbonate.

Afterripening of redbud seed is accompanied by or consists of a number of chemical and physical changes within the seed, more or less like those found in other seeds (*1*, *3*, *5*, *10*). Microchemical tests made on dormant redbud seed revealed large quantities of protein and fats, both in the endosperm and the embryo. Protein was particularly abundant in the cotyledons. Fats were abundant in the tips of the cotyledons and in the endosperm, yet present also in the radicle and the hypocotyl. Neither starch nor sugars were detected in a dormant seed. Oxidase was absent or inactive but the presence of peroxidase and catalase [3] was easily detected.

The embryo of a dormant seed is slightly acid (pH 6.7). The moisture content of an air-dry dormant seed including the seed coat is equal to approximately 10–10.5 percent of the fresh weight of the seed.

The microchemical tests at various stages of afterripening and during germination were made on seeds after 2, 4, 6, and 8 weeks of stratification preceded by treatment with concentrated sulfuric acid. Further statements pertaining to the changes in the amounts of various substances in seeds are based on the changes in the intensity of the re-

[3] Changes in catalase activity were determined quantitatively and are discussed later.

actions brought about by the application of proper reagents. A summary of the changes resulting from afterripening and germination is presented in table 6.

TABLE 6.—*Summary of various changes within the redbud seed as a result of afterripening and germination*

[Differences in number of crosses indicate relative changes in the amount of individual substances]

State of seed	Proteins	Fats	Starch	Reducing sugars	Oxidase	Peroxidase	Catalase [2]	pH	Moisture content (percent fresh weight)
Dormant [1]	XXX	XX	None	None	X	None	11.25	6.7	51.0
Stratified 2 weeks	XXX	XX	None	None	X	X	13.5	------	55.6
Stratified 4 weeks	XXX	X	None	Trace	X	X	17.0	6.3	55.8
Stratified 6 weeks	XXX	X	None	Trace	X	X	18.0	6.2	57.4
Seed germinated in stratification after 8 weeks	XX	X	XXX	X	XX	XX	23.4	6.5	63.5

[1] After sulphuric acid treatment and 24 hours of soaking.
[2] Cubic centimeters of O_2 evolved from 5 cc. of H_2O_2 in 10 minutes per 0.1 gm. of dry material.

As afterripening progressed the amount of proteins and fats decreased markedly, particularly the latter. However, after 6 weeks of stratification there still was a fair quantity of both in the endosperm and much protein in the embryo. Fats were still present in cotyledons and in the radicle but had disappeared from the hypocotyl.

The presence of reducing sugars was detected in seeds after 4 weeks of stratification. At that time sugars appeared in the cotyledons and in the radicle but were absent from the endosperm. The first appearance of reducing sugars in the endosperm was noted after 6 weeks of stratification, and even then only in very small amounts in the area adjacent to the embryo.

After 2 weeks of stratification oxidase appeared in the cotyledons, along their inner edges. After 4 weeks it became active throughout both the endosperm and the embryo. Peroxidase and catalase increased continuously throughout the period of afterripening. The reaction of the kernel changed from the original pH of 6.7 in a dormant seed to 6.3, 6.2, and 6.5 after 4, 6, and 8 weeks, respectively, of stratification. As a result of sulfuric acid treatment and soaking overnight, the moisture content of the seed increased from 10.3 percent to 32.6 percent of the weight of fresh material. Further soaking of the seed at room temperature (for a total of 24 hours) raised the water content of the seed to 51.0 percent. During 6 weeks of stratification, moisture in the seed remained more or less constant, fluctuating between 54.3 and 57.4 percent.

GERMINATION

MICROCHEMICAL CHANGES

Germination is accompanied by a sharp increase in moisture content of the seed, further reduction in the amount of proteins and fats, increase in the amount of reducing sugars and in the activity of oxidase, peroxidase, and catalase, a slight reduction in the acidity of the embryo, and a sudden appearance of a large quantity of starch (table 6). Starch is abundant in the cotyledons, the outer part of the hypocotyl, and in the tip of the radicle, although some appears also in the endosperm.

TEMPERATURE

Germination of afterripened redbud seed takes place at a rather wide range of temperatures, from 33° to 100° F., and probably even higher. Tests to determine the range of germination temperature and the optimum temperature were conducted on a total of 3,200 seeds, one-half of which, after being treated with sulfuric acid for 30 minutes, were stratified at 41° F. for 6 weeks and the other half for 8 weeks. Table 7 presents a summary of the progress of germination of 16 samples of seed (200 each) placed and kept in germinators at various temperatures, from 33° to 100° F.

TABLE 7.—*Germination of redbud seeds as affected by temperature*

Germination temperature (° F.)	Percentage germinations [1] after—			
	2 days	4 days	6 days	8 days
33°	0	0	0.7	0.7
42°	.25	.25	1.7	1.7
48°	.50	3.75	15.5	24.0
60°	25.2	55.7	84.2	91.0
70°	72.2	91.2	96.5	96.5
80°	87.5	90.7	91.2	91.2
90°	90.5	92.0	93.0	93.0
100°	29.0	45.5	50.5	53.7

[1] Average of 2 samples, one stratified for 6 weeks, the other for 8 weeks.

Within the range of 33° to 90° F. the rate of germination was directly affected by the temperature. Two days after the seeds had been set in germinators, 90.5 percent germinated at 90° while germination at 48° and below was less than 1 percent. After 4 days, germination of more than 90 percent was obtained at 70°, 80°, and 90°, and after 8 days more than 90 percent of the seeds germinated also at 60°. At 100° germination rose from 29.0 percent in 2 days to 53.7 percent in 8 days. The total germination (in 8 days) was highest at 70° (96.5 percent), although at that temperature it proceeded more slowly than at 80° or 90°. The temperature of 70° appears to be the optimum for germination of redbud seed. It happens to be the most favorable temperature also for growth of small seedlings. At a temperature of 60° and below seedlings grew more slowly than at 70°, although they apparently remained normal and sound. At a temperature of 80° and above, a certain degree of deterioration was evident among the seedlings. This was particularly evident at 100° at which temperature many of the seedlings rotted and others assumed an unhealthy abnormal appearance.

OXYGEN

Although afterripened redbud seeds germinate freely and rapidly in the normal atmosphere, and even under water when air is bubbled through it, an increase in the oxygen content of the atmosphere or the addition of it to water had an exceptionally stimulative effect on germination. Whereas germination of well afterripened seeds under normal conditions may be expected to be completed in a period of

6 to 8 days, afterripened seeds kept in pure oxygen were observed to germinate to the extent of 100 percent in 2 days.

In the investigation of the effect of oxygen on germination, a sample of seeds was placed in a cheesecloth bag suspended in a flask filled with pure oxygen. A few cubic centimeters of water were left at the bottom of the flask to prevent drying of the seeds and to provide the necessary moisture for germination. Another sample was placed in 100 cc. of distilled water through which oxygen was bubbled at the rate of 20 bubbles per minute. Checks for these treatments were a standard germination set-up (Petri dishes with moist cotton) and a sample of seeds placed under water. The original intention was to keep the seeds under treatment for 2 days and then to transfer them to the normal conditions of germination for comparison of their behavior with that of normally handled seeds. However, at the end of the first day, 1.5 percent of the seeds serving as a check in a Petri dish had germinated, while germination of seeds held in pure oxygen was 71.5 percent. At this time the seeds were removed from the oxygen flask and placed in moist cotton in a Petri dish in normal atmosphere. One day after this transfer germination of the oxygen-treated seeds rose to 90 percent while that of untreated seeds was only 13.5 percent. In a similar experiment carried out prior to the one just discussed, afterripened seeds kept in pure oxygen for 48 hours germinated 100 percent during that period as compared with 18 percent germination of seeds held in the normal atmosphere (table 8).

TABLE 8.—*Germination of afterripened seed under the influence of variations in the oxygen content of air and water*

Treatment	Percentage germination [1] after—				
	1 day	2 days	3 days	4 days	5 days
48 hours in 100 percent oxygen	10	100.0			
Check (normal atmosphere)		18.0			
24 hours in 100 percent oxygen	71.5	90.0		96.5	97.5
Check (normal atmosphere)	1.5	13.5		90.5	94.0
Set in nonaerated water	0	0	0	0	0
Set in water with O_2 bubbling through		92.0			

[1] Days from the time of setting of the experiment.

Bubbling oxygen through water in which afterripened seeds were placed had nearly the same effect as keeping the seeds in pure oxygen. Under these conditions germination of 92.0 percent was secured in 2 days. No germination took place among the seeds kept continuously under nonaerated water.

VITAMIN B_1

While treatment of nonafterripened seed with vitamin B_1 might have had a slight stimulative effect on the process of afterripening, the vitamin applied to afterripened seed was found to be entirely ineffective in stimulating germination. Afterripened seeds treated for 24 hours with a vitamin B_1 solution of 4 mg. per liter germinated almost at the same rate and to the same extent (95.5 percent) as the seeds that were not treated (97.5 percent).

EFFECT OF DRYING ON AFTERRIPENED SEEDS

During a normal procedure of afterripening, planting, or other handling of seeds, there is always a chance for them to dry more or less. Accidental drying of afterripened seeds may in some instances reduce germinability, throw seeds into a state of secondary dormancy (*3*), or even destroy their value completely by reducing their viability (*7*). Knowledge of the effect of drying on afterripened seeds seems to be of practical importance, suggesting the degree of care to be exercised while handling, storing, or shipping them.

Sixteen hundred seeds treated 30 minutes with sulfuric acid and kept in stratification for 55 days were placed in the laboratory at 75° to 85° F. and allowed to dry. From time to time samples of the seeds were tested for their ability to germinate. The results, presented in table 9, show that drying for 6 days had no effect on the continued ability of an afterripened embryo to resume growth. After 10 days of drying, germination fell off from the original of 96 percent to 90 percent, but when dry storage was extended to 30 days germination was reduced to 78 percent. Further extension of the dry storage to 60 and 90 days lowered germination to 4 and 6 percent, respectively.

TABLE 9.—*Effect of drying of afterripened seeds*[1] *on their ability to germinate*

Percent of dry storage (days)	Percent germination	Moisture content after drying (percent of total weight)	Period of dry storage (days)	Percent germination	Moisture content after drying (percent of total weight)
0 (check)	96	56.4	10	90	10.2
1	98	6.2	30	78	11.7
2	94	6.9	60	4	11.3
4	92	7.7	90	6	
6	98	9.0			

[1] All seeds treated 30 minutes with concentrated sufluric acid, then stratified 55 days.

A sharp decline in the germination ability of afterripened seeds kept dry for 60 and 90 days was not caused by an additional loss of moisture Under conditions of dry storage (temperature 75° to 85° F.) moisture equilibrium in seeds was reached after 24 hours of drying. Continued exposure of seeds to a temperature of 75° to 85° beyond that period resulted in an increase in moisture content of the seed which was probably due to the increase in the humidity of the surrounding atmosphere.

Drying afterripened seeds in an oven affected the viability of the seeds in various degrees, depending not only on the temperature of drying but also on the moisture content of the seeds at the time when the seeds were placed in the oven. When partially dried, redbud seeds were able to withstand considerable heat. In this investigation, seeds dried at room temperature for 4 days with moisture content reduced to 7.7 percent of the total weight of the seeds lost in viability from the original of 96 percent to 81 percent after being exposed to a temperature of 167° for 2 days.

The ability of partially dried seeds to withstand abnormally high temperatures is not uncommon. The present technique of seed extraction of coniferous species involves the use of dry kilns in which the temperature is raised to 110° and 120° F. (*14*).

CATALASE ACTIVITY

Catalase determinations in redbud seed were made by the method described by Davis (*4*) and adopted since by many other workers for measuring the activity of this enzyme in plant tissues. During the present investigation the entire seeds rather than kernels were tested for catalase activity because it is extremely difficult, if at all possible, to separate the seed coat from the perisperm. The amounts of various substances used in each catalase test were as follows: Water, 12 cc.; 3-percent solution of hydrogen peroxide, 5 cc.; plant material (seeds), approximately 600 mg.; $CaCO_3$ to equal the weight of plant material; and a pinch of pure quartz sand. In the following discussion catalase activity is expressed in terms of the number of cubic centimeters of oxygen evolved from 5 cc. of hydrogen peroxide in 10 minutes per 0.1 gm. of dry material of the seeds.

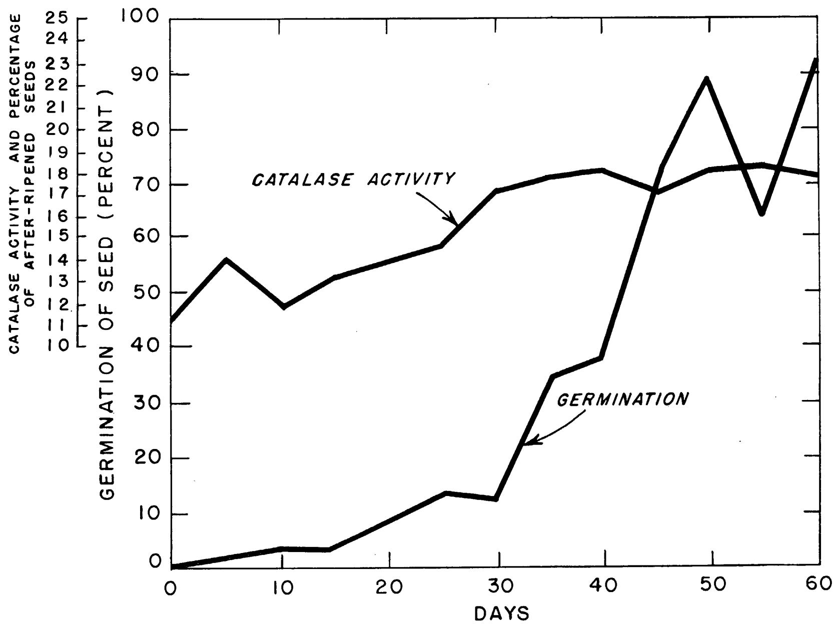

FIGURE 3.—Relation between degree of afterripening and catalase activity in redbud seed. Catalase activity is expressed in terms of cubic centimeters of oxygen given off in 10 minutes by C.01 gm. of dry material.

Catalase activity was determined in dormant dry seeds, seeds treated with concentrated sulfuric acid and soaked overnight, and seeds kept in stratification for various periods of time under conditions favoring afterripening. To obtain a good representative sample of the entire lot, 75 seeds were used in each test. On the days on which the tests were run, samples of seeds were placed in germinators to determine the degree of afterripening and the readiness of the seeds to germinate. The results of these tests are given in table 10 and figure 3.

Catalase activity in stratified redbud seeds increased with afterripening from 11.25 in dormant seed to an average of 17.95 in seeds more than 90 percent of which had completed their afterripening.

TABLE 10.—*Catalase activity and afterripening during stratification* [1]; *lot 38–B*

Period of stratification (days)	Lot 38-B_2		Lot 38-B_1	
	Catalase [2]	Germination	Catalase [2]	Germination
0	11.25	0	11.25	0
5	13.26	0	14.85	4.0
11	11.20	3.0	12.35	4.0
15	13.50	3.0	13.00	4.0
20	12.80		14.90	
25	15.00	17.0	14.30	10.0
30	17.00	11.0	17.30	14.0
35	18.05	34.0	17.55	34.0
40	18.00	50.0	18.36	26.0
46	17.50	89.0	16.70	55.0
50	18.60	95.0	17.65	84.0
55	18.85	81.0	17.50	47.0
60	18.25	95.0	17.65	88.0

[1] All seeds treated for 30 minutes with concentrated H^2SO^4.
[2] Catalase activity expressed in terms of the number of cubic centimeters of oxygen evolved in 10 minutes by 0.1 gra. of dry material.

The increase was very gradual, not entirely uniform, and not strongly correlated with the increase in germination. This suggests one of two possibilities: either catalase activity is more or less independent of the progress of afterripening, its intensity being simply controlled by the same external factors and in the same direction (though at

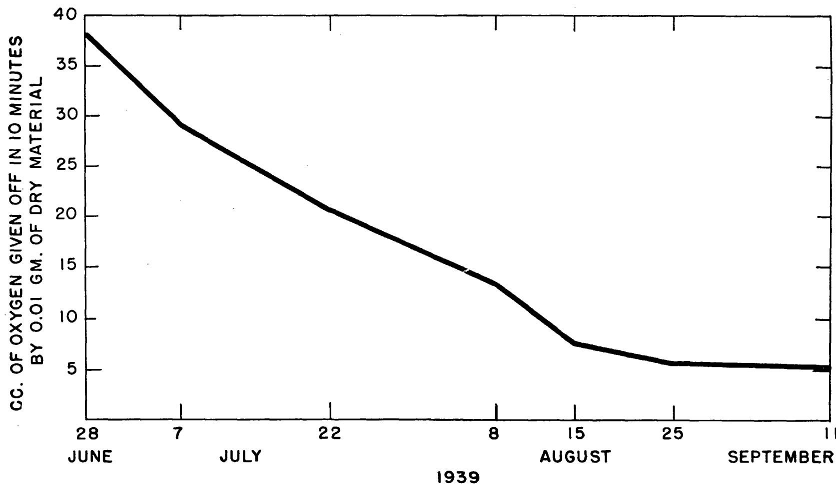

FIGURE 4.—Catalase activity as affected by the maturity of redbud seed. At the time of the two last tests (August 25 and September 11) the seeds were ripe.

different rates) as the afterripening; or, if changes of catalase activity are a part of the afterripening, they precede some other changes that are necessary for the completion of afterripening. The writer is inclined to consider the changes in catalase activity as a part of afterripening because of the close interrelationship between this and other processes on which the progress and the completion of the afterripening depend. Whether the relationship between catalase activity and the progress of other phases of afterripening is constant under all conditions and for all lots of seeds is, of course, open to question. Considerably more work is needed along this line.

In the course of the investigation catalase activity during the ripening process was also studied. Samples of seeds from a single plant were collected and catalase determinations made on various dates from June 28, 1939, to September 11 of the same year. On the former date the seeds were small, green, and soft. The two last tests (August 25 and September 11) were made on seeds which appeared to be completely ripe. The assumption that the seeds were ripe during the last two tests is substantiated by the results of catalase determinations, which showed that catalase activity attained a certain degree of stability.

As was expected (*11*), catalase activity fell off sharply with the ripening of the seed. At the time the seed reached maturity catalase activity was less than one-seventh of what it was 2 months earlier (fig. 4).

SUMMARY AND CONCLUSIONS

Delayed germination of seed of redbud (*Cercis canadensis* L.) is caused by the impermeability of the seed coat to water and by the dormancy of the embryo.

The seed coat can be rendered permeable to water by any of the standard treatments used with "hard-coated" seeds; namely, soaking of seed in concentrated sulfuric acid, hot water or boiling water bath, and mechanical scarification. The optimum treatment with sulfuric acid was of 30 to 35 minutes' duration

Extension of the storage of ripe dormant (unstratified) seeds to 15 months resulted in the lengthening of the required period of acid treatment from 30 to 45 minutes.

Stratification of acid-treated seed at low temperature (35° to 45° F.) was found to be effective in afterripening. The average period of stratification causing afterripening of not less than 90 percent of seeds varied between 5 and 8 weeks.

Holding afterripened seed in pure oxygen for 24 or 48 hours resulted in a marked increase in the rate of germination.

The optimum germination temperature of afterripened redbud seed was 70° F. Some germination occurred at a temperature as low as 33° F. Germination at 100° F. was slow and the seedlings produced and grown at that temperature either became unhealthy and abnormal in appearance or rotted completely.

Vitamin B_1 had no appreciable effect either on afterripening or germination

Ten days of dry storage of fully afterripened seed at 75° to 85° F. reduced the ability of the seed to germinate from the original of 96 percent to 90 percent.

Catalase activity increased during the progress of afterripening.

Catalase activity decreased steadily during the late stages of ripening of the seed.

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